Evaluation of Tc-99m Pyridoxal-Phenylalanine as a Hepatobiliary Imaging Agent. Part 1. Experimental Studies

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In this experimental study, the usefulness of a new radiotracer, Tc-99m pyridoxal-phenylalanine ("Tc-PPh"), is evaluated as a hepatobiliary imaging agent, and results are compared with those of conventional cholangiography. In the presence of a normal biliary tree or in cholelithiasis, information derived from conventional cholangiography is of better quality. The radiotracer technique, however, is very useful for the demonstration of cholopeptic bypass procedures, even in the presence of jaundice, and also in cases of intraperitoneal bile leakage.

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Visualization of the extrahepatic biliary tree is of paramount importance in certain clinical conditions. Though conventional radiologic methods are satisfactory in unjaundiced patients, their diagnostic contribution is minimal when serum bilirubin is elevated. This is true also when a biliary bypass operation has been performed.

Taplin et al. (*1*) introduced [^{131}I] rose bengal as a radioactive tracer for the hepatobiliary system; others followed with I-131 BSP, [^{123}I] rose bengal (*2*), Tc-99m penicillamine (*3*), Tc-99m dihydrothiotic acid (*4*), Tc-99m tetracycline (*5*), and Tc-99m mercaptoisobutyric acid (*6*).

Schiff's bases labeled with Tc-99m were introduced only recently (*7*), and they seem to have better biologic properties. In this study, Tc-99m-pyridoxal phenylalanine ("Tc-PPh") was tested for imaging of the extrahepatic biliary system.

MATERIALS AND METHODS

Preparation. One hundred mg of pyridoxal were mixed with 30 mg L-phenylalanine and 2 ml of water in a 10-ml bottle; the mixture was then buffered to pH 8 with 1 N NaOH, and 2 ml of [^{99m}Tc] sodium pertechnetate (= 20 mCi) added. The bottle was then sealed and autoclaved at 120°C and 15 lb/sq. in. for 30 min.

The preparation was then submitted to the standard radiobiologic tests, including electrophoresis in trisbarbital buffer (pH 8.8) (*7*) for 2 hr, which showed that its free pertechnetate (which migrated 11 cm from the origin) was less than 1%. The concentration of Tc-99m pyridoxal, migrating 3 cm from the origin, was negligible compared with the Tc-PPh, which moved to 1.5 cm.

Toxicity studies. Toxicity of untagged PPh was determined using 30 white mice weighing 20 ± 2 g each, five rabbits between 2,000 and 2,200 g, and two dogs weighing 20 and 23 kg.

A dose of 300 mg/kg was administered i.v. to each mouse by tail vein, 600 mg/kg to each rabbit, and 900 mg/kg to each dog. The experimental animals were followed up for 30 days by standard techniques. The doses administered were very well tolerated and the preparation was found to be sterile and pyrogen free. The rabbits were killed on the 31st day and the excised brain, heart, liver, gallbladder, stomach, duodenum, small intestine, lungs, spleen, kidneys, and skeletal muscles were examined histologically against two normal rabbits. No destruction or reaction was found in the specimens examined.

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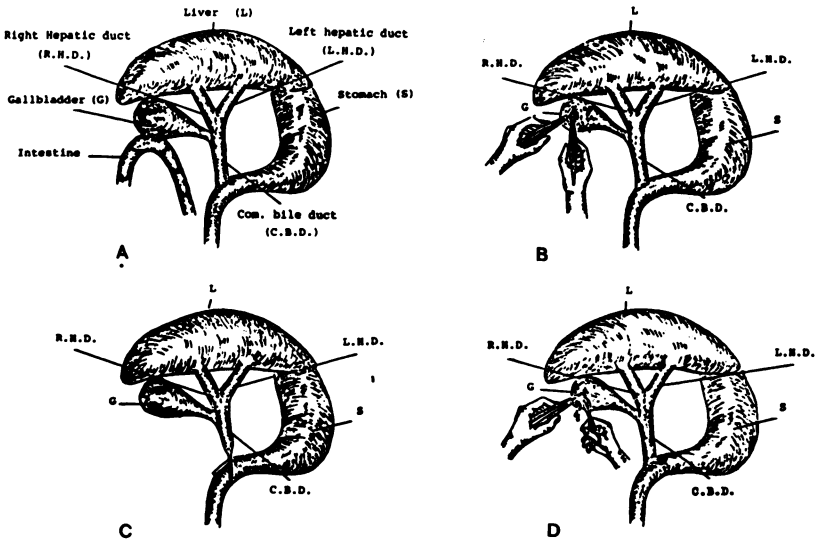

FIG. 1. (A) Side-to-side anastomosis of gallbladder and intestine. (B) Insertion of small gallstones in gallbladder. (C) Ligation of lower part of common bile duct using a tape. (D) Damage of gallbladder to produce experimental bile leakage.

Experimental studies. Fifty rabbits weighing from 2 to 4 kg were used, divided into the following five groups of ten:

1. Normal experimental animals.
2. A biliary bypass operation was performed.
3. Stones were placed into the gallbladder artificially.
4. Obstructive jaundice was induced surgically.
5. Intra-abdominal bile leakage was caused surgically.

After the experimental animals were anesthetized with i.v. pentothal (20 mg/kg), a middle laparotomy was carried out.

In Group 1 a sham operation was performed.

In Group 2 an anastomosis of the gallbladder to an intestinal loop was performed (Fig. 1a), thus constructing a biliary by-pass tract similar to that made in patients with obstruction of bile outflow to the duodenum. The gallbladder was anastomosed side-to-side to the jejunal loop in one layer, and a stoma 1.5 cm wide was constructed.

In Group 3 a small incision in the fundus of the gallbladder was made, and one to three small stones, taken from human operations and measuring from 2 × 2 mm to 2 × 5 mm, were inserted; the incision was then closed in one layer (Fig. 1b).

In Group 4 the common bile duct was tied near its lower end using cotton tape (Fig. 1c), thus obstructing the normal flow of bile.

As soon as bilirubin reached high levels (6–7 mg%), a Tc-PPh study of the biliary tree was carried out. The animals were then re-anesthetized, and a cholecystointestinal anastomosis similar to that performed in Group 2 was constructed. A new dose of Tc-PPh was then administered and configuration of the biliary tree re-evaluated.

Finally, in Group 5 a bile leak into the peritoneal cavity was produced by damaging the gallbladder (Fig. 1d), producing a hole 2 × 3 mm in its lower surface, near the fundus.

Half of the experimental animals used for each group were subjected to conventional radiographic techniques and the remaining half to scintigraphic procedures using Tc-PPh and a gamma camera.

RESULTS

Group 1. In five rabbits of this group, i.v. cholangiography was performed with an injection of 7 cc of "biligraphin forte." Opacification of the gallbladder was achieved within 1 hr.

The extrahepatic branches of the biliary tree were not clearly demonstrated, due to their small diameter (Fig. 2a).

Radiotracer images appeared in the following order: (a) at 1 min, the kidney silhouette, lasting from 10–15 min; (b) at 4 min, the liver and bladder; (c) at 10 min, the radiopharmaceutical appears in the duodenum; (d) at 10–15 min, the gallbladder appears; (e) at 20 min, the radiopharmaceutical advances into the small bowel (Fig. 2). The extrahepatic biliary tree was not visualized, but indirect information concerning the movement of the bile was deduced from the time of its passage to the intestine.

Group 2. Intravenous cholangiography proved unable to demonstrate the surgically constructed anastomosis between gallbladder and intestine in all five animals.

The silhouette of the route to the intestine was clearly demonstrated by the scintigraphic method (Figs. 3a and b), 10 min after the tracer was injected.

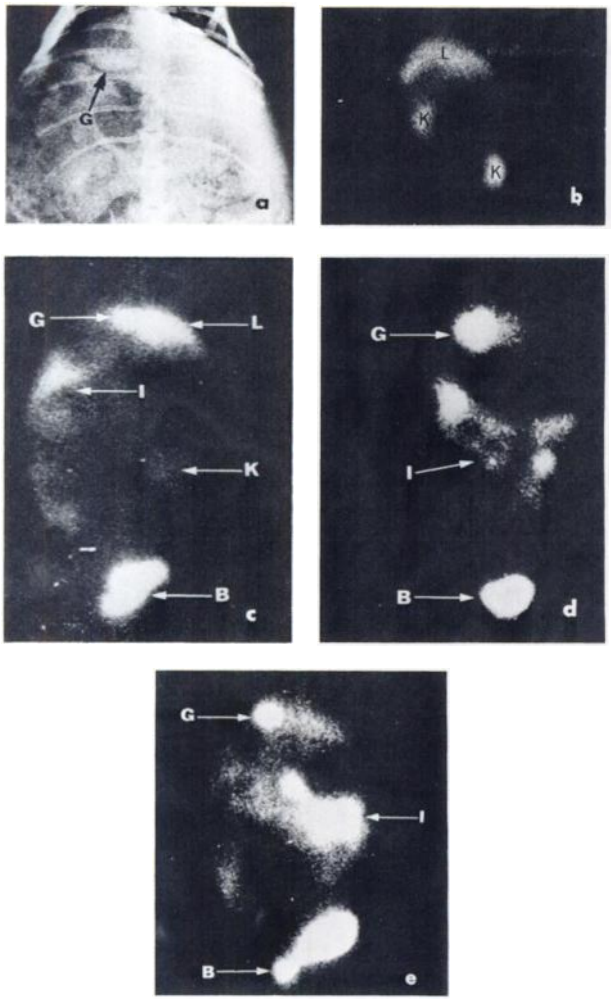

FIG. 2. (a) Cholangiographic opacification of the gallbladder (G) in the rabbit. (b) Silhouettes of a rabbit's liver (L) and kidneys (K) at the 4th min after i.v. administration of Tc-PPh tracer. (c) Ten minutes after the administration of Tc-PPh: the liver (L) gallbladder (G) and upper intestine (I) are demonstrated, while urinary bladder (B) is increasing in size and kidney shadows (K) begin to disappear. (d) Fifteen minutes following administration of Tc-PPh: gallbladder (G), intestines (I), and urinary bladder (B) are better demonstrated. The kidney shadows have gone. (e) The best image of the gallbladder (G) is obtained after 20–30 min (here 25 min), when tracer has moved farther down intestines (I). Urinary bladder (B) is also seen.

Group 3. The artificial cholelithiasis was demonstrated by the conventional radiographic techniques (Fig. 4a). On the other hand, the gamma images were not conclusive in this group (Fig. 4b).

Group 4. In jaundiced animals configuration of the biliary tree was not feasible with conventional i.v. cholangiography, but 5 min after the administration of the radiotracer the hepatic silhouette appeared. As time passes, the radioactivity of the bladder becomes predominant, and the radiopharmaceutical is then excreted, chiefly into the urine (Figs. 5a and b). In the icteric animals that were subjected to the cholopeptic anastomosis, the small intestine

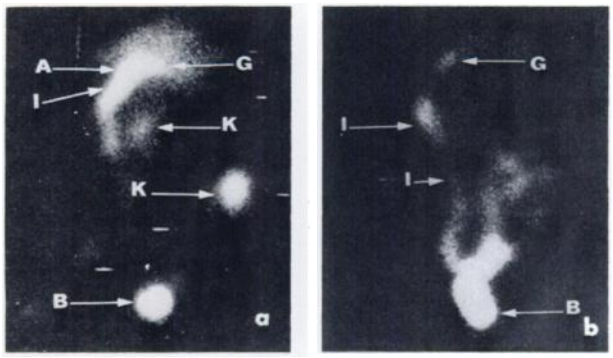

FIG. 3. (a) Ten minutes after i.v. injection of Tc-PPh, with an anastomosis between gallbladder (G) and intestine (I). The kidneys (K) and urinary bladder (B) are well demonstrated. (b) Rabbit as in Fig. 3a: 20 minutes after injection of Tc-PPh, the tracer is farther advanced in the intestine (I). Gallbladder (G) and the urinary bladder (B) are also demonstrated.

appeared 20 min after the i.v. injection of Tc-PPh, even when bilirubin levels were as high as 7.2 mg% (normal value 0.5 mg%).

Group 5. No signs of intra-abdominal leakage of bile were obtained with the conventional radiographic techniques.

Surprisingly enough the gamma images here were of great diagnostic value: the radiopharmaceutical was detected in the abdominal cavity, as illustrated in Fig. 6.

DISCUSSION

The purposes of this experimental project were: (a) to study the efficacy of Tc-PPh, which is a new radiopharmaceutical prepared at the Hellenic Center of Nuclear Research, as an hepatobiliary imaging agent, and (b) to compare results obtained by using the standard cholangiographic technique with those using the new agent.

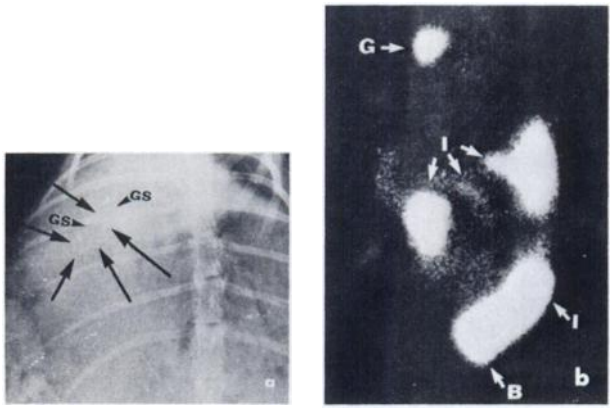

FIG. 4. (a) X-ray in experimental cholelithiasis. Gallstones (GS) are vaguely visible in gallbladder silhouette (long arrows). (b) Rabbit as in Fig. 4r 30 min after administration of Tc-PPh. Gallbladder (G) intestines (I) and urinary bladder (B) are demonstrated, but stones are not seen.

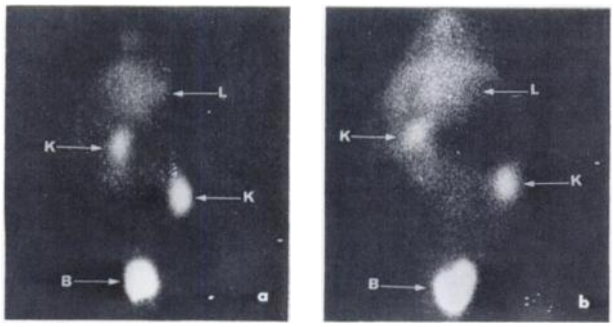

FIG. 5. Five (a) and 30 (b) min after administration of Tc-PPh in rabbit with obstructive jaundice. Shadows of liver (L), kidney (K), and urinary bladder (B) steadily appeared, whereas no intestinal radioactivity can be seen.

The radiobiologic tests of this compound proved it to be harmless. It is easily sterilized by standard techniques and proved to be pyrogen-free, since no reactions occurred. In the experimental rabbits, doses at least 1,000 times greater than those used in patients caused no histologically demonstrable damage in the brain, heart, liver, gallbladder, stomach, duodenum, small intestine, lungs, spleen, kidneys, or skeletal muscles.

Our findings show that the tracer is rapidly excreted from the polygonal cells of the liver, since clear images are obtained within 10 min after each administration.

In rabbits with a normal biliary tree, conventional cholangiography results in better imaging of the extrahepatic biliary tract.

Studies with Tc-PPh show that the order of appearance of the various organs in a scan is as follows: kidneys (1 min), liver (4 min), duodenum (10 min), gallbladder (10–15 min), small intestine (20 min).

A surprising finding was the demonstrability, by gamma imaging, of the gallbladder-to-intestine fistula produced experimentally in Group 2. This has potential clinical importance, since it is well known that i.v. cholangiography is useless in this situation.

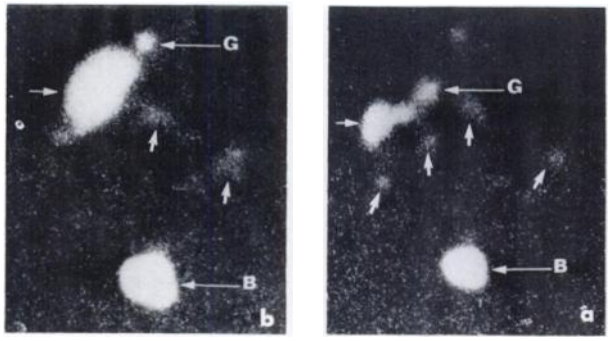

FIG. 6. Scintigraphic demonstration of intra-abdominal bile leakage, in two different animals, at 15th min. Gallbladder (G) and urinary bladder (B) are prominent, and the radiotracer is also seen free in abdominal cavity (arrows).

In cholelithiasis the imaging of the gallbladder was inconclusive, probably because of "see-through" radiation. In cases of obstructive jaundice, no signs of intestinal excretion were found, although the liver silhouette appeared at the 5th minute. The kidney and liver silhouettes remain for more than 1 hr due to recirculation of the agent. On the other hand, when a cholecystointestinal anastomosis was constructed, the tracer reached the intestine within 20 min, even in animals with high bilirubin levels. This makes the observations in Group 2 much more interesting from the viewpoint of clinical usefulness.

Finally, detection of the radiopharmaceutical in the peritoneal cavity in cases of bile leakage may prove the scan method to be unique in diagnosing this condition.

CONCLUSION

From our experimental study the following conclusions have been drawn:

1. With a normal extrahepatic biliary tree, conventional cholangiography is superior to scintigraphy.
2. In cholelithiasis, conventional cholangiography is superior to scintigraphy.
3. Gamma imaging with Tc-PPh is of high diagnostic value in assessing patency of a biliary-intestinal anastomosis.
4. In cases of obstructive jaundice, the scintigraphic method is of indirect value because of the maintenance of liver and kidney images for more than 1 hr, due to recirculation of the tracer.
5. Gamma imaging is uniquely useful for the demonstration of intraperitoneal bile leakage.

It is concluded that Tc-99m pyridoxal-phenylalanine is a valuable diagnostic agent in demonstrating certain disturbances of the extrahepatic biliary tract. The method is safe and rapid; results can be obtained within 10 min. It is simple, since no special preparation is required, and diagnostic information is easy to interpret.

Further experience with the method is important, so that more detailed information may be available.

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